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A NEW RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SACUBITRIL AND VALSARTAN IN ITS BULK AND TABLET DOSAGE FORM AND ITS FORCE DEGADATION STUDIES AS PER ICH

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ABSTRACT
A New method was established for simultaneous estimation of Sacubitril And Valsartan by RP-HPLC method. Chromatographic separations were carried using Inertsil ODS (4.6 x 150 mm, 5µm) column with a mobile phase composition of 0.1% OPA buffer and Acetonitrile(50:50) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 with PDA detector 2996 at wavelength 271 nm. The retention time for Sacubitril and Valsartan were 2.119 and 2.730 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 12-60 µg/ml for Sacubitril and 13-65µg/ml for Valsartan respectively. For accuracy The total recovery was found to be 100.30% and 100.22% for Sacubitril and Valsartan. The LOD and LOQ for Sacubitril was found to be 2.96 and 9.96 and LOD and LOQ for Valsartan was found to be 2.98 and 9.98. The force degradation studies were performed and the results are within the limits. The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Sacubitril and Valsartan in pharmaceutical dosage form.

KEYWORDS: Sacubitril, Valsartan, RP-HPLC, Simultaneous estimation.

INTRODUCTION
Sacubitril is an antihypertensive drug used in combination with valsartan.[1]The combination drug valsartan/sacubitril, marketed under the brand name Entresto, is a treatment for heart failure.[2] Sacubitril is a prodrug that is activated to sacubitril at by de-ethylation via esterases.[3] Sacubitrilat inhibits the enzyme neprilysin. The most common adverse reactions with sacubitril plus valsartan included hypotension, hyperkalemia, cough, dizziness, and renal failure. Sacubitrilis chemically (S)-5-[(4- phenylphenyl)methyl] pyrrolidin-2-one. Structre shown in fig.1. Sacubitril Slightly soluble in water, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

Valsartan is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack.[4] Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure.[5] The drug binds to angiotensin type I receptors (AT1), working as an antagonist. This mechanism of action is different than the ACE inhibitor drugs, which block the conversion of angiotensin I to angiotensin II. Since valsartan acts at the receptor, it can provide more complete angiotensin II antagonism since angiotensin II is generated by other enzymes as well as ACE.(B) Most common side effects include dizziness, low blood pressure, and diarrhea. Valsartan is chemically (2S)-3-methyl-2-(N-{[2'-(2H-1,2,3,4-tetrazole-5-yl)biphenyl-4-yl]methyl}pentanamido) butanoic acid. Structre shown in fig.2. Valsartan Soluble in Acetonitrile, practically insoluble in water also soluble in methanol.

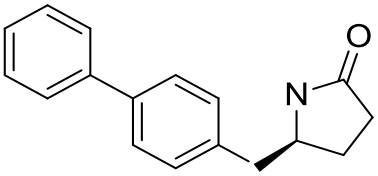

Figure 1: Structure of Sacubitril.

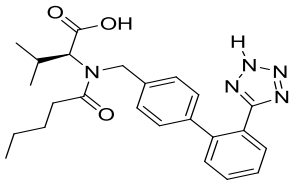

Figure 2: Structure of Valsartan.

Literature survey shows that a number of methods have been reported for estimation of Sacubitril And Valsartan individually or in combination with other drugs Those are HPLC Methods,[6-16] LC-MS Methods,[17] Ultraviolet spectrophotometry,[18-19] However, there is only one HPLC method is reported for the simultaneous estimation of these drugs in combined dosage forms.[20] But it not explained force Degradation study. I got better results than already published one.

The aim of the present study was A New Rp-Hplc Method For Simultaneous Estimation Of Sacubitril And Valsartan In Its Bulk And Tablet Dosage Form And Its Force Degadation Studies As Per Ich.

MATERIALS AND METHODS

Chemicals and Reagents: Sacubitril and Valsartan were obtained as a gift sample from Natco laboratories, Hyderabad. KH_2PO_4 was analytical grade supplied by Finer chemical LTD, Mumbai, Orthophosphoric acid (Merck), Acetonitrile (Molychem, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 271 nm with an Inertsil ODS (4.6 x 150 mm, 5µm) dimensions at ambient temperature. The optimized mobile phase consists of 0.1% OPA and Acetonitrile in the ratio of 50:50 v/v. Flow rate was maintained at 1 ml/min and run time for 6 min.

Preparation of solutions

Preparation of buffer

Take 1 ml of ortho phosphoric acid in 1000 ml volumetric flask and make up to the mark with HPLC water and sonicate for 15minutes then then filter through 0.45µ filter under vacume filteration.

Preparation of mobile phase

Mix a mixture of above buffer 500 mL (50%) and 500 mL of Acetonitrile HPLC (50%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration

The diluents

The Mobile phase was used as the diluent.

Preparation of standard stock solution

Accurately weigh and transfer 12&13mg of Sacubitril & Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 3.0ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample stock solution

Accurately weigh and transfer equivalent to 12 &13mg of Sacubitril & Valsartan sample (Tablet powder) into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). Further pipette 1.0 ml of Sacubitril & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 3.0 ml of Sacubitril & Valsartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject 20µL of the standard, sample into the chromatographic system and measure the areas for the Sacubitril & Valsartan peaks and calculate the %Assay by using the formulae.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.4ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 5 µL of standard into Inertsil ODS (4.6 x 150 mm, 5µm), the mobile phase of composition 0.1% OPA buffer and acetonitrile in the (50:50) was allowed to flow through the column at a flow rate of 0.4ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Sacubitril & Valsartan in their tablet dosage form. The result obtained for Sacubitril & Valsartan was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Sacubitril and Valsartan in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 12µg/ml to 60µg/ml of Sacubitril and 13µg/ml to 65µg/ml of Valsartan. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard

solutions into chromatographic system. Calculate the Amount found and Amount added for Sacubitril and Valsartan and calculate the individual recovery and mean recovery values. The resulte are shown in table 4.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 5.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2ml/min. The Organic composition in the Mobile phase was varied from 50% to 50%.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Force degradation Studies
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Sacubitril and Valsartan using the proposed method.

Preparation of Stock
Accurately weigh and transfer 10&10mg of Sacubitril& Valsartan working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 1.0 ml of Sacubitril& Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Hydrolytic Degradation under Acidic Condition
Pipette 3.0 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic Degradation under Alkaline Condition
Pipette 3.0 ml of above solution into a 10ml volumetric flask into and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal Induced Degradation
Sacubitril and Valsartan sample was taken in petridish and kept in Hot air oven at 1100 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative Degradation
Pipette 3.0 ml above stock solution-2 into a 10ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide added and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSION

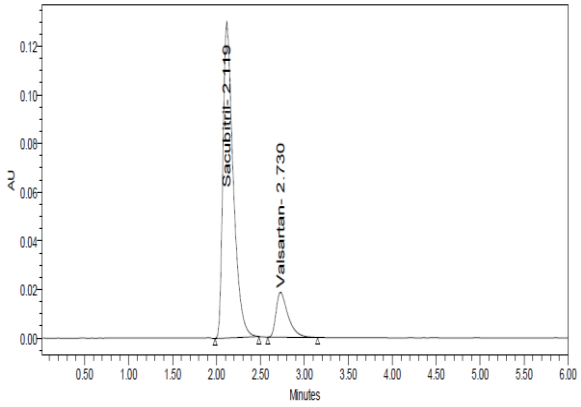

Figure 3: Standard chromatogram.

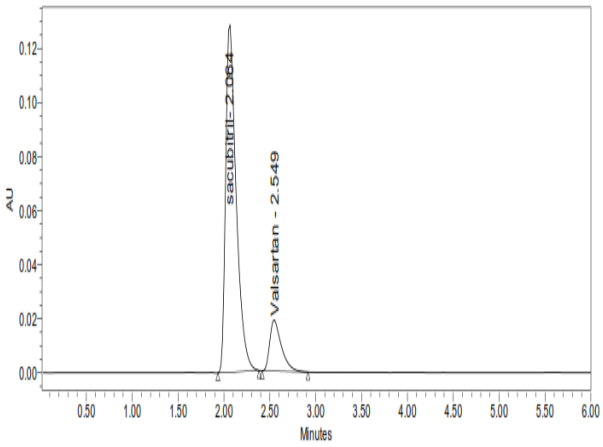

Figure 4: Sample chromatogram.

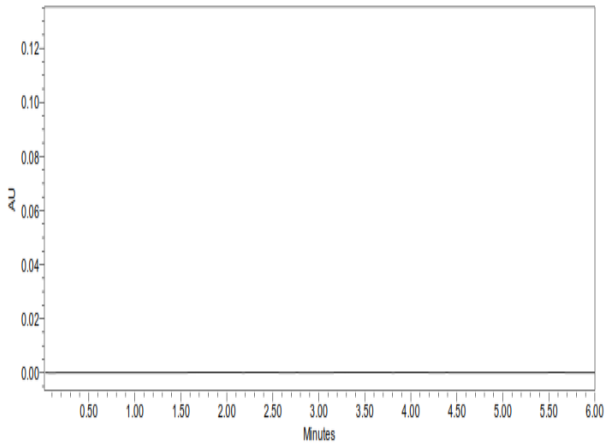

Figure 5: Blank chromatogram.

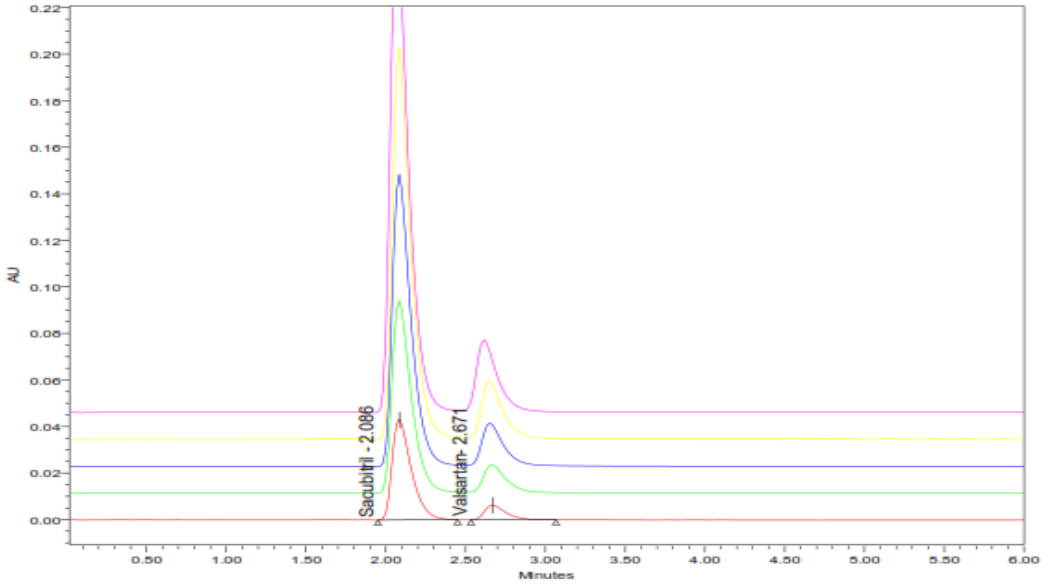

Figure 6: Linearity Overlay Chromotogram.

Table 1: System suitability parameters.

Parameters	Sacubitril	Valsartan
Retention time	2.119	2.730
USP Plate count	2524.84	3177.99
USP Tailing	1.6	1.4

Assay Calculation for Sacubitril

$$\frac{1043362}{1050652} * \frac{12}{10} * \frac{1}{10} * \frac{3}{10} * \frac{10}{130.5} * \frac{10}{1} * \frac{10}{3} * \frac{261}{24} * \frac{99.8}{100} * 100 = 99.11$$

Assay Calculation for Valsartan

$$\frac{170732}{173068} * \frac{13}{10} * \frac{1}{10} * \frac{3}{10} * \frac{10}{130.5} * \frac{10}{1} * \frac{10}{3} * \frac{261}{26} * \frac{99.8}{100} * 100 = 98.45$$

Table 2: Assay results for Sacubitril and Valsartan.

	Label Claim (mg)	% Assay
Sacubitril	24	98.26
Valsartan	26	98.55

Table 3: Linearity results for Sacubitril and Valsartan.

Sacubitril		Valsartan	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Area**
12	360303	13	59045
24	692178	26	114337
36	1019720	39	168147
48	1343531	52	222495
60	1679118	65	276005
Correlation coefficient	0.999	Correlation coefficient	0.999

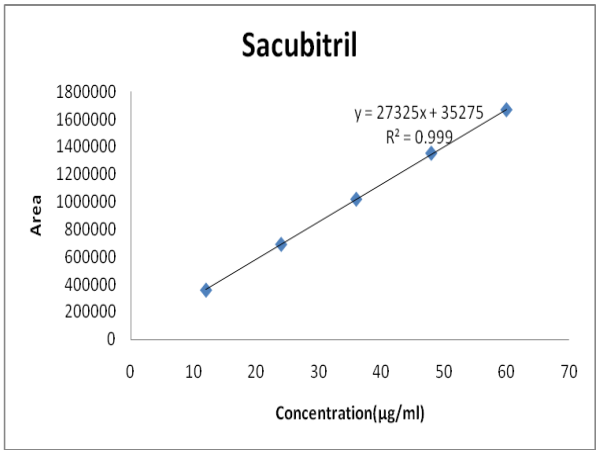

Figure 4: Linearity graph for Sacubitril.

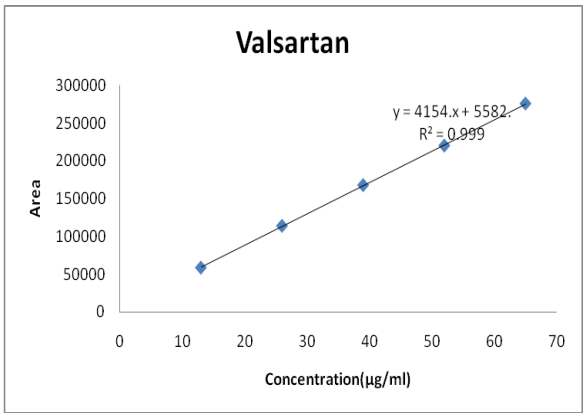

Figure 5: Linearity graph for Valsartan.

Table 4: Precision results for Sacubitril and Valsartan

Injection	Sacubutril Area	Valsartan Area
Injection-1	1023945	168040
Injection-2	1027796	167914
Injection-3	1026845	170372
Injection-4	1036375	169848
Injection-5	1020865	167068
Average	1027165.2	168648.4
Standard Deviation	5817.7	1397.9
%RSD	0.6	0.8

Table 5: Ruggedness results for Sacubitril and Valsartan

Injection	Sacubitril Area	Valsartan Area
Injection-1	1003903	164423
Injection-2	1018214	165485
Injection-3	1012117	166719
Injection-4	1018518	165469
Injection-5	1009168	166045
Injection-6	1020368	165226
Average	1013714.4	165561.0
Standard Deviation	6435.6	774.2
%RSD	0.6	0.5

Table 6: Degradation results for Sacubitril and Valsartan.

Parameters	Sacubitril	% Degraded	Valsartan	% Degraded
Standard	1050652		173068	
Acid	937570	10.8	151073	12.71
Base	920335	12.41	152390	11.95
Peroxide	1026845	2.33	166068	4.05
Thermal	1019720	3.0	168147	2.9
Photo	1018518	3.19	166045	4.06

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Sacubitril and Valsartan in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Sacubitril and Valsartan in pure and its pharmaceutical dosage forms.

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